BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12357

Oxidative stress, redox signalling and endothelial dysfunction in ageing-related neurodegenerative diseases: a role of NADPH oxidase 2

Sarah Cahill-Smith & Jian-Mei Li

Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK

Correspondence
Professor Jian-Mei Li MD, PhD, Faculty of Health and Medical Sciences, University of Surrey, AY building, Guildford, Surrey GU2 7XH, UK.
Tel.: +44 1483 686475
Fax: +44 1483 686401
E-mail: j.li@surrey.ac.uk

Keywords
ageing, Alzheimer's disease, mild cognitive impairment, NADPH oxidase 2, Parkinson's disease, redox signalling

Received
28 October 2013
Accepted
12 February 2014
Accepted Article Published Online
19 February 2014

Chronic oxidative stress and oxidative damage of the cerebral microvasculature and brain cells has become one of the most convincing theories in neurodegenerative pathology. Controlled oxidative metabolism and redox signalling in the central nervous system are crucial for maintaining brain function; however, excessive production of reactive oxygen species and enhanced redox signalling damage neurons. While several enzymes and metabolic processes can generate intracellular reactive oxygen species in the brain, recently an $O_2^{\cdot-}$-generating enzyme, NADPH oxidase 2 (Nox2), has emerged as a major source of oxidative stress in ageing-related vascular endothelial dysfunction and neurodegenerative diseases. The currently available inhibitors of Nox2 are not specific, and general antioxidant therapy is not effective in the clinic; therefore, insights into the mechanism of Nox2 activation and its signalling pathways are needed for the discovery of novel drug targets to prevent or treat these neurodegenerative diseases. This review summarizes the recent developments in understanding the mechanisms of Nox2 activation and redox-sensitive signalling pathways and biomarkers involved in the pathophysiology of the most common neurodegenerative diseases, such as ageing-related mild cognitive impairment, Alzheimer's disease and Parkinson's disease.

Introduction

With a growing ageing population, the number of people with neurodegenerative diseases is steadily increasing [1]. Ageing-related neurodegenerative diseases are a group of diseases characterized by the progressive loss of neurons, leading to dysfunction of the central nervous system, and include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease [2–5]. According to the World Health Organization, there are nearly 35.6 million people living with dementia, and this number is expected to double by 2030. Although the exact mechanisms behind the damage and loss of neurons in these detrimental diseases remain unknown, increasing evidence has suggested that an ageing-associated increase in the production of reactive oxygen species (ROS) causes central nervous system oxidative stress, microvascular dysfunction and neuronal damage [6, 7].

ROS include both free radicals, e.g. superoxide ($O_2^{\cdot-}$) and the hydroxyl radical ($OH^{\cdot}$), and nonradicals, e.g. hydrogen peroxide (H_2O_2) [8]. ROS can be produced by a number of different means, including mitochondria [9], uncoupled nitric oxide (NO) synthase [10], xanthine oxidase [11] and NADPH oxidase [12]. They are short lived and can react quickly with biomolecules to alter their activities. Low levels of intracellular ROS are now recognized to have an important role in the maintenance of normal cellular function and redox signalling [13]. However, an excess of ROS results in oxidative stress, which involves damage to cellular components, such as lipids, proteins and nucleic acids, and leads to the loss of biological function [13]. Although there are several enzymes and metabolic processes that can generate intracellular ROS, recently an $O_2^{\cdot-}$-generating enzyme called NADPH oxidase 2 (Nox2), which is constitutively expressed in a variety of cell types in the brain, including cerebral vascular endothelial cells, has

emerged as a major source of oxidative stress in ageing-related neurodegenerative diseases.

The vascular endothelium is a major target of oxidative stress, and there is a close link between vascular endothelial dysfunction and cognitive impairment [14–17]. Nox2 is highly expressed in the endothelium, and endothelial oxidative stress due to Nox2 activation in response to environmental challenges increases cerebrovascular permeability and promotes leucocyte adhesion and central nervous system inflammation. Indeed, altered microvascular endothelial function has been recognized as a key factor of advanced ageing and cognitive impairment [14–17]. Several previous reviews have discussed the importance of oxidative stress in the biology of ageing [18–20]; therefore, the present review focuses on the recent development in understanding the mechanism of Nox2 activation, as well as the role of Nox2-derived oxidative stress and redox signalling in the development of age-related neurodegenerative diseases, such as mild cognitive impairment (MCI), Alzheimer's disease and Parkinson's disease.

Mechanism of Nox2 activation and the principle of Nox2 redox signalling

Nox2 (a multicomponent enzyme) was originally discovered in circulating phagocytic cells [21]. It consists of a membrane-bound catalytic core called flavocytochrome b_{558}, consisting of $gp91^{phox}$ (β subunit), $p22^{phox}$ (α subunit) and several cytosolic regulatory subunits called $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ and rac1 [22]. Nox2 is normally dormant in resting phagocytes, but when facing a pathogen challenge (or phagocyte activation) the cytosolic subunits translocate to the membrane and associate with cytochrome b_{558} to activate $gp91^{phox}$. Activated $gp91^{phox}$ then uses NADPH as an electron donor to convert molecular oxygen to $O_2\cdot^-$ (so-called oxidative burst) and to eliminate the pathogen [23]. In the past 15 years, several homologues of $gp91^{phox}$ have been identified and renamed Nox to represent NADPH oxidase. Presently, the Nox family includes seven members (Nox1–5 and Duox 1–2), with the original phagocytic $gp91^{phox}$ being named Nox2. Each Nox is encoded for by a separate gene [8]. In this review, Nox2 refers to the NADPH oxidase complex containing $gp91^{phox}$ as the catalytic subunit. Nox1 was initially found in colonic epithelial cells [24], Nox3 is highly expressed in the inner ear [25], Nox4 was first identified in the kidney [26, 27], Nox5 is primarily expressed in testis, spleen and lymph nodes [28], and Duox1 and Duox2 are primarily expressed in the thyroid gland [29, 30].

Vascular tissue is rich in Nox isoforms. For example, Nox1 is highly expressed in smooth muscle cells, Nox2 is highly expressed in endothelial cells, and Nox4 and Nox5 are detected in both smooth muscle and endothelial cells [31, 32]. The central nervous system also expresses Nox isoforms, with Nox2 and Nox4 being the most prominent isoforms detected in a variety of cell types, including neurons, microglia and astrocytes [33–37]. Among these Nox isoforms, Nox2 has been shown to be an important source of $O_2\cdot^-$ production in cerebral arteries [38] and plays a major role in cerebrovascular diseases [39]. In a mouse model of stroke, treatment with apocynin or deletion of Nox2 reduced infarct volume in the cortex and subcortex after cerebral ischaemia–reperfusion in association with reduced $O_2\cdot^-$ production [40, 41]. However, in the absence of reperfusion, ROS production by Nox2 seemed not to have a significant role in the pathophysiology of cerebral ischaemia [42].

Table 1

Redox signalling pathways in mouse models of neurodegenerative diseases

Strain	Model	Signalling pathways examined	Reference
SAMP8	Ageing	MDA, NO, GPx	[146]
C57Bl/6N Sim	Ageing	MDA, 8-OHdG	[72]
C57Bl/6	HFLD	Protein carbonyl	[79]
SAMP8	Ageing	Protein carbonyl, TBARS	[147]
C57Bl/6N Sim	Ageing	Protein carbonyl, MDA	[148]
Balb/C	Ageing	GSH	[149]
FVB/N	Tg2576	8,12-iso-$iPF_{2\alpha}$-VI	[150]
C57BL/6J	LPS	TNFα, IL-1β, Iba-1, MCP-1	[132]
C57BL/6J	MPTP	iNOS, 3-NT, 4-HNE	[151]
C57BL/6J	MPTP	MAC-1, Iba-1, ED-1, 8-OHdG, 3-NT, iNOS, MPO	[134]
C57BL/6J	LPS	TNFα, IL-6, KC, IL-12, NO, nitrite, p-p38	[143]
C57BL/6J	MPTP	MAC-1, iNOS, TNFα	[143]
C57BL/6J	MPTP	iNOS, IL-1β, TNFα, CD11b, GFAP, NF-κB	[152]
C57BL/6J	MPTP	ASK1, p-MKK4, p-JNK, GSH, Daxx, DJ-1	[142]
C57BL/6J	MPTP	4-HNE, 3-NT, GSH, GSSG	[135]
C57BL/6J	MPTP	Nitrite	[131]
C57BL/6J	MPTP	MAC-1, protein carbonyl	[133]
C57BL/6	MPTP	Bax, p53	[145]
C57BL/6J	MPTP	p-JNK, p-MKK4	[141]

3-NT, 3-nitrotyrosine; 4-HNE, 4-hydroxynonenal; 8,12-iso-$iPF_{2\alpha}$-VI, isoprostane; 8-OHdG, 8-Hydroxy-2′-deoxyguanosine; ASK1, apoptosis signal-regulating kinase 1; Bax, Bcl-2-associated X protein; CD11b, cluster of differentiation molecule 11b; Daxx, death-associated protein; DJ-1, Parkinson's disease protein; ED-1, clone ED1 CD68 microglial/macrophage marker; GFAP, glial fibrillary acidic protein; GPx, glutathione peroxidise; GSH, glutathione; GSSG, oxidized glutathione; HFLD, high fat lard diet; Iba-1, ionized calcium binding adapter molecule 1; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-12, interleukin-12; iNOS, inducible nitric oxide synthase; KC, keratinocyte-derived chemokine; LPS, lipopolysaccharide; MAC-1, macrophage antigen complex-1; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MPO, myeloperoxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NFκB, nuclear factor kappa B; NO, nitric oxide; p-MKK4, phospho-dual specificity mitogen-activated protein kinase kinase 4; p-JNK, phospho-c-jun N-terminal kinase; p-p38, phospho-p38 mitogen-activated protein kinase; p53, tumour suppressor protein; TBARS, thiobarbituric acid reactive substance; Tg2576, mouse model of Alzheimer's disease amyloidosis; TNFα, tumour necrosis factor alpha.

Table 2

Redox signalling pathways in human neurodegenerative diseases

Diagnosis	Signalling pathways examined	Ref.
MCI	LPO	[89]
MCI	MDA	[87]
MCI	MDA, GPx, catalase	[85]
MCI	SOD, GPx, MDA	[95]
MCI	MDA, nitrite and nitrate, GSSG	[86]
MCI	3-NT	[91]
MCI	Protein carbonyl	[153]
MCI	4-HNE	[90]
MCI	Protein carbonyl, TBARS, MDA	[84]
MCI	Vitamins A, C and E, uric acid, SOD, GPx	[94]
MCI	8,12-iso-iPF$_{2\alpha}$-VI	[88]
Alzheimer's disease	MDA	[87]
Alzheimer's disease	MDA, GPx, catalase	[85]
Alzheimer's disease	SOD, GPx, MDA	[95]
Alzheimer's disease	MDA, nitrite and nitrate, GSSG	[86]
Alzheimer's disease	iNOS, eNOS, nNOS, p53, UCP2,4,5, mitochondrial complexes I–V, PPARα, δ, γ	[124]
Alzheimer's disease	3-NT	[154]
Alzheimer's disease	Protein carbonyl, TBARS, MDA	[84]
Alzheimer's disease	8-OH-adenine, 5-OH-cytosine, 5-OH-uracil, 8-OH-guanine	[155]
Alzheimer's disease	3-NT	[156]
Alzheimer's disease	Vitamin A, C and E, uric acid, SOD, GPx	[94]
Alzheimer's disease	8,12-iso-iPF$_{2\alpha}$-VI	[88]
Alzheimer's disease	p-p38	[123]
Parkinson's disease	8-OHdG	[157]
Parkinson's disease	p-p38, p-JNK	[144]
Parkinson's disease	NF-κB	[152]
Parkinson's disease	p-Src, p-HSP27, p-JNK	[158]
Parkinson's disease	Glutathione reductase	[159]
Parkinson's disease	TBARS	[160]
Parkinson's disease	p-ERK1/2, p-p38, p-JNK	[140]

3-NT, 3-nitrotyrosine; 4-HNE, 4-hydroxynonenal; 8,12-iso-iPF$_{2\alpha}$-VI, isoprostane; 8-OHdG, 8-Hydroxy-2′-deoxyguanosine; eNOS, endothelial nitric oxide synthase; GPx, glutathione peroxidise; GSSG, oxidized glutathione; iNOS, inducible nitric oxide synthase; LPO, lipid hydroperoxide; MCI, mild cognitive impairment; MDA, malondialdehyde; NFκB, nuclear factor kappa B; nNOS, neuronal nitric oxide synthase; p-ERK, phospho-extracellular signal-regulated kinase; p-HSP27, phospho-heat shock protein 27; p-JNK, phospho-c-jun N-terminal kinase; p-p38, phospho-p38 mitogen-activated protein kinase; p-Src, phospho-proto-oncogene tyrosine-protein kinase; p53, tumour suppressor protein; PPAR, peroxisome proliferator-activated receptor; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; UCP, uncoupling protein.

Although Nox2 in nonphagocytic cells shares structural similarity with the phagocytic Nox2, the regulatory mechanism of Nox2 activation in nonphagocytic cells is completely different from that in phagocytic cells [43]. We have reported that Nox2 in resting endothelial cells is already partly pre-assembled and continuously generates very low levels of $O_2^{\cdot -}$, which is involved in redox regulation of normal cellular functions [43, 44]. Phosphorylation of the p40^{phox} subunit has been found to be involved in maintaining the basal Nox2 activity in nonphagocytic cells [45]. However, Nox2 in nonphagocytic cells can respond to a variety of stimulants to become activated and cause oxidative stress to cells and organs. Rac1 regulation of Nox2 activity and protein phosphorylation represent two of the important mechanisms in Nox2 activation [46, 47]. Our recent studies have shown that serine phosphorylation of p47^{phox} is a prerequisite for Nox2 activation in response to stimulation by tumour necrosis factor α (TNFα) [48, 49], angiotensin II [50, 51] and the protein kinase C activator phorbol 12-myristate 13-acetate [49]. Furthermore, we have discovered that the phosphorylation of p47^{phox} at serine residues S303, S304 and S379 plays a vital role in initiating Nox2 activation and $O_2^{\cdot -}$ production under pathological conditions [52].

In response to cellular challenges, such as growth factors, inflammatory cytokines, cell stress such as ischaemia–reperfusion and cytotoxic reagents, Nox2 is activated, and the resulting increase in $O_2^{\cdot -}$ production plays a key role in somatic cellular senescence and ageing [53–55]. In terms of central nervous system inflammation, Nox2 in microglial cells (the phagocytes in the brain) is activated in response to neurotoxic stimulation, and excessive ROS production causes neuronal damage. However, microglial cells can respond to neuronal damage and become further activated. This activation can be long lived, self perpetuating and eventually kill the neuron [37].

The primary product of Nox2 is $O_2^{\cdot -}$, which is short lived and can react with several different molecules to form other ROS, such as H_2O_2, $OH^{\cdot}$, hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) [8]. Therefore, it is possible that Nox2 redox signalling occurs through these secondary products rather than through $O_2^{\cdot -}$ itself [13]. Most proteins involved in cellular signalling contain cysteine residues or protein-bound metals, which are targets of ROS modification [56]. Thiol chemistry plays an important role in maintaining cellular redox homeostasis and therefore plays a key role in directing the redox signalling pathways [13]. Redox signalling involves both reversible and irreversible protein reactions. A good example of a reversible reaction is protein phosphorylation and dephosphorylation, and a lot of protein kinases and phosphatases are redox-sensitive targets (for details see end of paragraph). ROS-induced irreversible reactions include protein degradation or protein *S*-nitrosylation. Oxidation of lipids, proteins, DNA bases and the sugar backbones of DNA and RNA are mainly irreversible [13]. In terms of neurodegeneration, protein *S*-nitrosylation has been reported to be involved in redox-mediated post-translational modification of proteins and causes synaptic damage and brain cell death [57]. Recent studies from others and ourselves have found that the most common downstream targets of Nox2-derived ROS are mitogen-activated protein kinases (MAPKs), such as the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK [48, 52, 58, 59]; protein kinase B [60–63]; phosphoinositide 3-kinase [64]; nuclear factor κB (NFκB) [65]; TNFα receptor-associated factor 4 [48]; and signalling molecules involved in cell apoptosis, such as p21^{cip1} and p53 [66].

Oxidative stress and redox signalling in ageing-related mild cognitive impairment

Oxidative stress has been seen to play a major role in the mammalian ageing process and is the result of an imbalance between pro-oxidant and antioxidant levels. There has been extensive research looking at levels of various oxidative stress biomarkers during ageing; for example, protein carbonyls to detect protein oxidation [67–69], malondialdehyde (MDA) to detect lipid peroxidation [68, 70, 71] and 8-hydroxy-2′-deoxyguanosine to detect DNA oxidation [72, 73]. Levels of tissue ROS have also been found to increase with ageing [74, 75], along with decreases in various antioxidants, such as vitamins A and C, and antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase [76–78].

During the normal ageing process, a progressive decline in cognitive function linked with multi-organ oxidative stress has been observed in numerous experimental studies, implicating that cognitive impairment may be associated with ageing-related oxidative damage in the brain. For example, increased $O_2^{\cdot -}$ production in the brain was found in ageing mice (24 months old) in association with significant cognitive impairment [74]. More recently, another study reported increases in ROS production, lipid peroxidation and nucleic acid oxidation in ageing mice (23 months old) in correlation with a decline in learning and memory [72]. A high-fat lard diet further exacerbated cognitive decline in aged mice (24 months old), and this was associated with high levels of oxidative stress in the hippocampus [79].

Mild cognitive impairment (MCI) is generally characterized as a memory deficit abnormal for the individual's age. Clinical diagnosis criteria include the following: (i) memory complaint, preferably corroborated by an informant; (ii) objective memory impairment for age and education; (iii) normal general cognitive function; (iv) intact activities of daily living; and (v) no evidence of dementia [80]. MCI is generally seen as a transitional state between normal cognitive ageing and dementia and is frequently associated with the development of Alzheimer's disease, with annual conversion rates of MCI to Alzheimer's disease of ~10–15% [81, 82]. Ageing is a strong risk factor for both MCI and Alzheimer's disease, with prevalence for MCI estimated to be 1% at age 60 years and increase to 42% by age 85 years and prevalence for Alzheimer's disease estimated to be 1% at age 60 years and increase to 25% by age 85 years [83]. As previously mentioned, age-related increases in oxidative stress have been widely reported, and evidence is emerging for the role of oxidative stress in the pathogenesis of MCI and Alzheimer's disease.

High levels of oxidative stress related to cognitive decline have been observed. For example, in patients with MCI, increases in protein carbonyl content and MDA were found in the superior and middle temporal gyri, which were indicative of protein oxidation and lipid peroxidation, respectively [84]. Increased peripheral MDA has been found in erythrocytes, serum and plasma [85–87] of patients with MCI. Other markers of lipid peroxidation include: the isoprostane 8,12-iso-iPF$_{2\alpha}$-VI, which has been found to be significantly increased in the plasma, cerebrospinal fluid and urine of MCI patients [88]; lipid hydroperoxide, which has been found to be increased in the serum of MCI patients [89]; and 4-hydroxy-2-nonenal, which has been found to be increased in the hippocampus and inferior parietal lobules of MCI patients [90].

Nitrosative stress is also seen in ageing. Increased 3-nitrotyrosine levels were found in the hippocampus and inferior parietal lobule of aged patients (88 ± 3.8 years old) with MCI, which is indicative of protein nitration [91]. One of the major causes of protein nitration is the reaction with peroxynitrite, which is a potent oxidizing and nitrating agent produced by the reaction of superoxide and nitric oxide. Increased expression of inducible nitric oxide synthase has been found with ageing in mice (22 months old), which led to increased levels of nitric oxide that can react readily with the abundant superoxide present [92].

MCI is associated not only with an increase in pro-oxidants, but also with a decrease in antioxidants. Several studies have shown a decrease in levels of non-enzymatic antioxidants, including vitamins A, C and E and uric acid, and a decrease in activity of antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, in serum and plasma of patients with MCI [93–95]. In fact, a report has found a direct correlation between antioxidant depletion and cognitive deterioration [86].

Nox2 is a major source of oxidative stress involved in the development of ageing-related MCI. Nox2 expression has been found to be increased in the superior and middle temporal gyri of patients with MCI and in the brain of ageing mice (23 months old) [96, 97]. Aged mice overexpressing the amyloid precursor protein (12–15 months old) exhibited signs of cognitive decline and had significantly increased levels of Nox2-derived superoxide production in the brain [98]. There is a linear relationship between cognitive decline and Nox2 activity [99, 100]. Moreover, increased expression of the Nox2 subunits p47^{phox} and p67^{phox} has also been reported in the temporal cortex of MCI patients, which again correlated strongly with a decline in cognition [101]. These findings strongly support a substantial role for Nox2-derived oxidative stress in the development of cognitive impairment.

Oxidative stress and redox signalling in Alzheimer's disease

Alzheimer's disease is the most common form of dementia and is characterized by progressive memory loss, cognitive impairment, aphasia (language disturbance), apraxia (impaired ability to carry out motor activities despite intact

motor function), agnosia (failure to recognize or identify objects despite intact sensory function) and disturbance in executive functioning (i.e. planning, organizing, sequencing and abstracting) [102, 103]. Alzheimer's disease is associated with accumulation of amyloid plaques and neurofibrillary tangles constituted by highly phosphorylated tau proteins in the brain, and the loss of neurons from the hippocampus and cerebral cortex [104]. Nox2 activation in brain tissue and Nox2-derived oxidative damage has emerged as an important mechanism in the pathogenesis of Alzheimer's disease [105, 106].

Alzheimer's disease is strongly linked with accumulation of the protein β-amyloid, and there is a close relationship between the levels of β-amyloid and Nox2 activity [100, 107]. Cerebral amyloid angiopathy characterized by deposition of β-amyloid in the cerebrovasculature is present in 80–100% of Alzheimer's disease cases [108]. β-Amyloid is toxic to brain endothelial cells, leading to the loss of blood–brain barrier integrity. Increased expression of Nox2 and oxidative stress were found in activated microglia surrounding β-amyloid-laden capillaries from patients with capillary cerebral amyloid angiopathy [108, 109]. In addition, neuronal cell viability was shown to be significantly decreased in the presence of β-amyloid, and this effect was attenuated in the presence of a Nox2 inhibitor, apocynin [97]. However, it is important to note that it is still disputable whether the mode of action of apocynin is as an antioxidant or as a Nox2 inhibitor [110–114]. Apocynin has been shown to have a neuroprotective effect in mouse models of ischaemia and amyotrophic lateral sclerosis; however, in a model of Alzheimer's disease it did not improve behavioural or neuropathological deficits despite causing a reduction in oxidative stress in the cerebral cortex [115–117]. In a mouse model of Alzheimer's disease with overexpression of the amyloid precursor protein, the level of superoxide production in the brain was significantly increased in association with neurovascular dysfunction and behavioural deficits, and these were significantly attenuated in mice with Nox2 deficiency [98]. Nox2-derived oxidative stress has also been found to play a major role in mediating β-amyloid protein-induced neuronal death and neurovascular dysfunction [118, 119].

A variety of stimuli, such as bacterial components, inflammatory cytokines, β-amyloid peptide and other neurotoxins, have been shown to activate Nox2 and induce ROS production in brain tissue [37, 120]. Increased ROS production activates downstream signalling pathways, such as ERK1/2 and the phosphorylation of cytosolic phospholipase $A_2\alpha$ [121]. Overactivation of cytosolic phospholipase $A_2\alpha$ is believed to contribute to the pathogenesis of a number of neurodegenerative diseases, including Alzheimer's disease [122]. Other redox-sensitive MAP kinases, such as p38 MAPK, have also been found to be upregulated in the brains of patients with Alzheimer's disease [123]. More recently, an increase in the expression of p53, a redox-sensitive apoptosis-related molecule, has been found in the brain tissue of patients with Alzheimer's disease, and this was associated with increased nitric oxide synthase and Nox1 and Nox3 expression [124]. The amyloid precursor protein involved in Alzheimer's disease pathogenesis has been seen rapidly to activate JNK, ERK and p38 MAPK in rat microglial cells in association with increased inducible nitric oxide synthase expression [125]. Stimulation of rat pheochromocytoma (PC12) cells with β-amyloid activated the NFκB, ERK and p38 MAPK pathways, which was associated with increases in intracellular ROS production, the levels of expression of apoptotic proteins p53, Bcl-2-associated X protein and caspase-3, and downregulation of the anti-apoptotic protein Bcl-2 [126]. β-Amyloid has also been found to activate the NFκB pathway by selectively inducing the nuclear translocation of p65 and p50 subunits in human neurons [127].

Oxidative stress and redox signalling in Parkinson's disease

Parkinson's disease is a movement disorder characterized by bradykinesia, rigidity, resting tremor and postural instability and is associated with the progressive loss of dopaminergic neurons from the substantia nigra region in the midbrain [15, 128, 129]. The aetiology and underlying mechanisms of Parkinson's disease are still under investigation but increasing evidence is emerging for the involvement of Nox2-derived ROS and oxidative stress. Ageing is a strong risk factor for oxidative stress and the development of Parkinson's disease. Nox2 is highly expressed in microglial cells [130] and to a lesser extent in dopaminergic neurons [33]. Nox2 has been found along with degeneration of dopaminergic neurons and is implicated in the development of Parkinson's disease [131, 132].

Several studies carried out in mice have used administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which selectively destroys the nigrostriatal dopaminergic pathway, as a model of Parkinson's disease [131, 133–135]. One such study showed that Nox2 expression was upregulated both in MPTP-treated mice and in patients with Parkinson's disease, indicating a crucial role of Nox2 in the MPTP-induced loss of dopaminergic neurons [133]. A ROS scavenger, ethyl pyruvate, was found to improve MPTP-induced motor deficits by attenuating Nox2 activation, ROS production, microglial activation and neuronal loss. The mechanism involved was found to be in part due to the inhibition of $p47^{phox}$ phosphorylation and the subsequent binding of $p47^{phox}$ to $gp91^{phox}$ [134].

Studies of other mouse models have also been carried out, and a transgenic mouse model of Parkinson's disease has recently been created with mutations in leucine-rich repeat kinase 2 (LRRK2), which is the most

common genetic cause of Parkinson's disease. These LRRK2^{R1441G} transgenic mice exhibit the key characteristics of Parkinson's disease, including age-dependent and levodopa-responsive slowness of movement along with impaired dopamine release [136]. A recent study found that treatment of these transgenic mice with diapocynin, a Nox2 inhibitor, alleviated symptoms of early Parkinson's disease; in particular, motor co-ordination and balance [137]. However, due to the high concentration of diapocynin required to inhibit hydroethidine oxidation to 2-hydroxyethidium, the exact mechanism behind the effects of diapocynin in these mice remains unknown [137]. As mentioned previously, the mechanism of apocynin and diapocynin action needs further investigation.

The molecular mechanisms regulating dopaminergic neuronal cell death are not yet fully understood, but evidence suggests an important role of ROS. Increased ROS production and MAPK activation, including p38 MAPK and ERK1/2, has been seen to be involved in dopaminergic cell death [138]. In an experimental model of Parkinson's disease, increased phosphorylation of JNK and p38 MAPK was evident as well as increased apoptosis in human dopaminergic SH-SY5Y cells [139]. Likewise, activation of ERK1/2, JNK and p38 MAPK was found in brains of patients with Parkinson's disease [140]. In the MPTP animal model of Parkinson's disease, increased levels of JNK and MKK4 phosphorylation were found to be associated with activation of apoptosis signal-regulating kinase 1 and a decrease in DJ-1, the Parkinson's disease-associated neuroprotective protein [141, 142]. MAPK-activated protein kinase-2 (MK2) is a downstream target of p38 MAPK. A recent study has shown that neurodegeneration can be prevented in a mouse model of lipopolysaccharide-induced Parkinson's disease by eliminating MK2 [143]. In that study, it was also discovered that increased production of inflammatory mediators, such as TNFα, keratinocyte-derived chemokine, interleukin-6 and nitric oxide, with lipopolysaccharide administration was associated with decreased levels of tyrosine hydroxylase-positive neurons, and this was

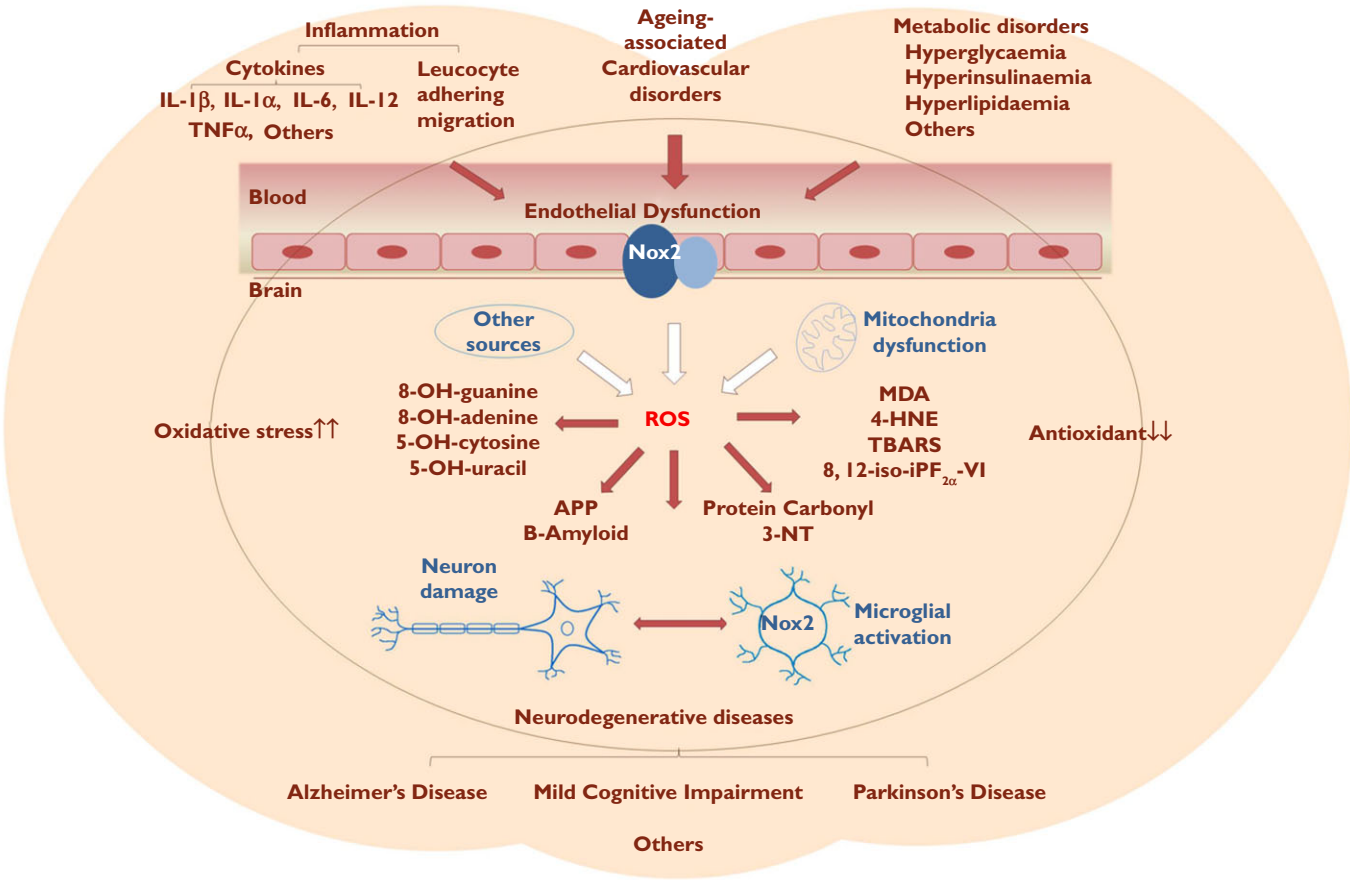

Figure 1

Schematic summary of factors involved in the brain oxidative stress and oxidative biomarkers in the pathogenesis of neurodegenerative diseases. Abbreviations are as follows: 3-NT, 3-nitrotyrosine; 4-HNE, 4-hydroxynonenal; 8,12-iso-iPF$_{2\alpha}$-VI, isoprostane; APP, amyloid precursor protein; IL-1α, interleukin-1 alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-12, interleukin-12; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substance; TNFα, tumour necrosis factor alpha

attenuated in MK2$^{-/-}$ mice [143]. In mice treated with MPTP, p38 MAPK activation was found to induce p53 phosphorylation and nuclear translocation, and this subsequently increased activity of the p53-upregulated modulator of apoptosis and Bcl-2-associated X protein [144]. Inhibition of p53 activity improved motor function, reduced damage to nigrostriatal dopaminergic neurons and prevented the increase in Bcl-2-associated X protein in MPTP-treated mice, and this in turn protected against MPTP-mediated cell death in human primary neurons [144, 145]. Put together, these data strongly support the pivotal roles for Nox2 and oxidative activation of p53 in the pathophysiology of Parkinson's disease.

Conclusion

The pathophysiology of the most common neurodegenerative diseases, such as ageing-related MCI, Alzheimer's disease and Parkinson's disease, involves multiple factors that cause the deterioration of the central nervous system throughout the ageing process. Existing endothelial dysfunction and metabolic disorders contribute to the exacerbation of the clinical symptomatology. Although precise cellular and molecular mechanisms underlying these diseases are still largely unknown, oxidative stress due to the activation of Nox2 in the neurovascular endothelium, microglia, neurons and other brain cells represents one of the key features and common determinants responsible, at least in part, for the pathological process of these diseases. Figure 1 is a schematic summary of factors discussed in this review that are involved in the pathogenesis of common neurodegenerative diseases. The redox signalling pathways discussed in this article are listed in Table 1 for experimental animal studies and in Table 2 for clinical human studies. Understanding the mechanism of Nox2 activation and its redox signalling pathways will help the discovery of novel specific drug targets to slow down the progress and, eventually, to treat these detrimental neurodegenerative diseases.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: SC-S had support from Age UK for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

Sarah Cahill-Smith was supported by an RIA research scholarship from Age UK.

REFERENCES

1 Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing. Nature 2008; 451: 716–9.

2 Johri A, Beal MF. Antioxidants in Huntington's disease. Biochim Biophys Acta 2012; 1822: 664–74.

3 D'Amico E, Factor-Litvak P, Santella RM, Mitsumoto H. Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. Free Radic Biol Med 2013; 65C: 509–27.

4 Garcia-Garcia A, Zavala-Flores L, Rodriguez-Rocha H, Franco R. Thiol-redox signaling, dopaminergic cell death, and Parkinson's disease. Antioxid Redox Signal 2012; 17: 1764–84.

5 Feng Y, Wang X. Antioxidant therapies for Alzheimer's disease. Oxid Med Cell Longev 2012; 2012: 472932–48.

6 Schrag M, Mueller C, Zabel M, Crofton A, Kirsch WM, Ghribi O, Squitti R, Perry G. Oxidative stress in blood in Alzheimer's disease and mild cognitive impairment: a meta-analysis. Neurobiol Dis 2013; 59: 100–10.

7 Sultana R, Perluigi M, Butterfield DA. Oxidatively modified proteins in Alzheimer's disease (AD), mild cognitive impairment and animal models of AD: role of Abeta in pathogenesis. Acta Neuropathol 2009; 118: 131–50.

8 Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 2007; 87: 245–313.

9 Starkov AA. The role of mitochondria in reactive oxygen species metabolism and signaling. Ann N Y Acad Sci 2008; 1147: 37–52.

10 Porasuphatana S, Tsai P, Rosen GM. The generation of free radicals by nitric oxide synthase. Comp Biochem Physiol Part C Toxicol Pharmacol 2003; 134: 281–9.

11 Kelley E, Khoo N, Hundley N, Malik U. Hydrogen peroxide is the major oxidant product of xanthine oxidase. Free Radic Biol 2010; 48: 493–8.

12 Dworakowski R, Anilkumar N, Zhang M, Shah AM. Redox signalling involving NADPH oxidase-derived reactive oxygen species. Biochem Soc Trans 2006; 34: 960–4.

13 Forman HJ, Fukuto JM, Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. Am J Physiol Cell Physiol 2004; 287: C246–56.

14 Gates PE, Strain WD, Shore AC. Human endothelial function and microvascular ageing. Exp Physiol 2009; 94: 311–6.

15 Gazewood JD, Richards DR, Clebak K. Parkinson disease: an update. Am Fam Physician 2013; 87: 267–73.

16 Leszek J, Sochocka M, Gąsiorowski K. Vascular factors and epigenetic modifications in the pathogenesis of Alzheimer's disease. J Neurol Sci 2012; 323: 25–32.

17 Dickstein DL, Walsh J, Brautigam H, Stockton SD, Gandy S, Hof PR. Role of vascular risk factors and vascular dysfunction in Alzheimer's disease. Mt Sinai J Med 2010; 77: 82–102.

18 Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239–47.

19 Lenaz G. Role of mitochondria in oxidative stress and ageing. Biochim Biophys Acta 1998; 1366: 53–67.

20 Hadley EC, Lakatta EG, Morrison-Bogorad M, Warner HR, Hodes RJ. The future of aging therapies. Cell 2005; 120: 557–67.

21 Babior BM, Lambeth JD, Nauseef W. The neutrophil NADPH oxidase. Arch Biochem Biophys 2002; 397: 342–4.

22 Rae J, Newburger PE, Dinauer MC, Noack D, Hopkins PJ, Kuruto R, Curnutte JT. X-Linked chronic granulomatous disease: mutations in the CYBB gene encoding the gp91-phox component of respiratory-burst oxidase. Am J Hum Genet 1998; 62: 1320–31.

23 Wientjes FB, Segal AW. NADPH oxidase and the respiratory burst. Semin Cell Biol 1995; 6: 357–65.

24 Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1. Nature 1999; 401: 79–82.

25 Banfi B, Malgrange B, Knisz J, Steger K, Dubois-Dauphin M, Krause KH. NOX3, a superoxide-generating NADPH oxidase of the inner ear. J Biol Chem 2004; 279: 46065–72.

26 Geiszt M, Kopp JB, Varnai P, Leto TL. Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci U S A 2000; 97: 8010–4.

27 Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, Hattori M, Sakaki Y, Sumimoto H. A novel superoxide-producing NAD(P)H oxidase in kidney. J Biol Chem 2001; 276: 1417–23.

28 Bánfi B, Molnár G, Maturana A, Steger K, Hegedûs B, Demaurex N, Krause KH. A Ca^{2+}-activated NADPH oxidase in testis, spleen, and lymph nodes. J Biol Chem 2001; 276: 37594–601.

29 De Deken X, Wang D, Many MC, Costagliola S, Libert F, Vassart G, Dumont JE, Miot F. Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J Biol Chem 2000; 275: 23227–33.

30 Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D, Virion A. Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cdnas. J Biol Chem 1999; 274: 37265–9.

31 Montezano AC, Burger D, Ceravolo GS, Yusuf H, Montero M, Touyz RM. Novel Nox homologues in the vasculature: focusing on Nox4 and Nox5. Clin Sci (Lond) 2011; 120: 131–41.

32 Takac I, Schroder K, Brandes RP. The Nox family of NADPH oxidases: friend or foe of the vascular system? Curr Hypertens Rep 2012; 14: 70–8.

33 Choi D-H, Cristóvão AC, Guhathakurta S, Lee J, Joh TH, Beal MF, Kim Y-S. NADPH oxidase 1-mediated oxidative stress leads to dopamine neuron death in Parkinson's disease. Antioxid Redox Signal 2012; 16: 1033–45.

34 Cooney SJ, Bermudez-Sabogal SL, Byrnes KR. Cellular and temporal expression of NADPH oxidase (NOX) isotypes after brain injury. J Neuroinflammation 2013; 10: 155.

35 Infanger DW, Sharma RV, Davisson RL. NADPH oxidases of the brain: distribution, regulation, and function. Antioxid Redox Signal 2006; 8: 1583–96.

36 Sorce S, Krause K-H. NOX enzymes in the central nervous system: from signaling to disease. Antioxid Redox Signal 2009; 11: 2481–504.

37 Block ML. NADPH oxidase as a therapeutic target in Alzheimer's disease. BMC Neurosci 2008; 9: (Suppl 2): S8.

38 Miller AA, Drummond GR, De Silva TM, Mast AE, Hickey H, Williams JP, Broughton BRS, Sobey CG. NADPH oxidase activity is higher in cerebral versus systemic arteries of four animal species: role of Nox2. Am J Physiol Heart Circ Physiol 2009; 296: H220–5.

39 Chrissobolis S, Faraci FM. The role of oxidative stress and NADPH oxidase in cerebrovascular disease. Trends Mol Med 2008; 14: 495–502.

40 Jackman KA, Miller AA, De Silva TM, Crack PJ, Drummond GR, Sobey CG. Reduction of cerebral infarct volume by apocynin requires pretreatment and is absent in Nox2-deficient mice. Br J Pharmacol 2009; 156: 680–8.

41 De Silva TM, Brait VH, Drummond GR, Sobey CG, Miller AA. Nox2 oxidase activity accounts for the oxidative stress and vasomotor dysfunction in mouse cerebral arteries following ischemic stroke. PLoS One 2011; 6: e28393.

42 Kim HA, Brait VH, Lee S, De Silva TM, Diep H, Eisenhardt A, Drummond GR, Sobey CG. Brain infarct volume after permanent focal ischemia is not dependent on Nox2 expression. Brain Res 2012; 1483: 105–11.

43 Li J-M, Shah AM. Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. J Biol Chem 2002; 277: 19952–60.

44 Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. Am J Physiol Integr Comp Physiol 2004; 287: R1014–30.

45 Fan LM, Teng L, Li JM. Knockout of p47 phox uncovers a critical role of p40 phox in reactive oxygen species production in microvascular endothelial cells. Arterioscler Thromb Vasc Biol 2009; 29: 1651–6.

46 Di-Poï N, Fauré J, Grizot S, Molnár G, Pick E, Dagher MC. Mechanism of NADPH oxidase activation by the Rac/Rho-GDI complex. Biochemistry 2001; 40: 10014–22.

47 El-Benna J, Dang PM, Gougerot-Pocidalo MA, Marie JC, Braut-Boucher F. p47phox, the phagocyte NADPH oxidase/NOX2 organizer: structure, phosphorylation and implication in diseases. Exp Mol Med 2009; 41: 217–25.

48 Li JM, Fan LM, Christie MR, Shah AM. Acute tumor necrosis factor alpha signaling via NADPH oxidase in microvascular endothelial cells: role of p47phox phosphorylation and binding to TRAF4. Mol Cell Biol 2005; 25: 2320–30.

49 Li J-M, Mullen AM, Yun S, Wientjes F, Brouns GY, Thrasher AJ, Shah AM. Essential role of the NADPH oxidase subunit $p47^{phox}$ in endothelial cell superoxide production in response to phorbol ester and tumor necrosis factor-α. Circ Res 2002; 90: 143–50.

50 Li JM, Shah AM. Mechanism of endothelial cell NADPH oxidase activation by angiotensin II. Role of the p47phox subunit. J Biol Chem 2003; 278: 12094–100.

51 Li JM, Wheatcroft S, Fan LM, Kearney MT, Shah AM. Opposing roles of $p47^{phox}$ in basal versus angiotensin II-stimulated alterations in vascular O_2^- production, vascular tone, and mitogen-activated protein kinase activation. Circulation 2004; 109: 1307–13.

52 Teng L, Fan LM, Meijles D, Li J-M. Divergent effects of $p47^{phox}$ phosphorylation at S303-4 or S379 on tumor necrosis factor-α signaling via TRAF4 and MAPK in endothelial cells. Arterioscler Thromb Vasc Biol 2012; 32: 1488–96.

53 Lener B, Kozieł R, Pircher H, Hütter E, Greussing R, Herndler-Brandstetter D, Hermann M, Unterluggauer H, Jansen-Dürr P. The NADPH oxidase Nox4 restricts the replicative lifespan of human endothelial cells. Biochem J 2009; 423: 363–74.

54 Schilder YDC, Heiss EH, Schachner D, Ziegler J, Reznicek G, Sorescu D, Dirsch VM. NADPH oxidases 1 and 4 mediate cellular senescence induced by resveratrol in human endothelial cells. Free Radic Biol Med 2009; 46: 1598–606.

55 Jeon SM, Lee S-J, Kwon TK, Kim K-J, Bae Y-S. NADPH oxidase is involved in protein kinase CKII down-regulation-mediated senescence through elevation of the level of reactive oxygen species in human colon cancer cells. FEBS Lett 2010; 584: 3137–42.

56 Forman HJ, Torres M, Fukuto J. Redox signaling. Mol Cell Biochem 2002; 234-235: 49–62.

57 Akhtar MW, Sunico CR, Nakamura T, Lipton SA. Redox regulation of protein function via cysteine S-nitrosylation and its relevance to neurodegenerative diseases. Int J Cell Biol 2012; 2012: 463756.

58 Tickner J, Fan LM, Du J, Meijles D, Li J, Sciences M. Nox2-derived ROS in PPARγ signaling and cell-cycle progression of lung alveolar epithelial cells. Free Radic Biol Med 2011; 51: 763–72.

59 Fan L, Sawbridge D, George V, Teng L, Bailey A, Kitchen I, Li JM. Chronic cocaine-induced cardiac oxidative stress and mitogen-activated protein kinase activation: the role of Nox2 oxidase. J Pharmacol Exp Ther 2009; 328: 99–106.

60 Hingtgen SD, Tian X, Yang J, Dunlay SM, Peek AS, Wu Y, Sharma RV, Engelhardt JF, Davisson RL. Nox2-containing NADPH oxidase and Akt activation play a key role in angiotensin II-induced cardiomyocyte hypertrophy. Physiol Genomics 2006; 26: 180–91.

61 Liu L, Yang Z, Xu Y, Li J, Xu D, Zhang L, Sun J, Xia S, Zou F, Liu Y. Inhibition of oxidative stress-elicited AKT activation facilitates PPARγ agonist-mediated inhibition of stem cell character and tumor growth of liver cancer cells. PLoS ONE 2013; 8: e73038.

62 Du J, Fan LM, Mai A, Li J-M. Crucial roles of Nox2-derived oxidative stress in deteriorating insulin receptor and endothelial function in dietary obesity of middle-aged mice. Br J Pharmacol 2013; 170: 1064–77.

63 Thakur S, Du J, Hourani S, Ledent C, Li J-M. Inactivation of adenosine A_{2A} receptor attenuates basal and angiotensin II-induced ROS production by Nox2 in endothelial cells. J Biol Chem 2010; 285: 40104–13.

64 Bae YS, Sung JY, Kim OS, Kim YJ, Hur KC, Kazlauskas A, Rhee SG. Platelet-derived growth factor-induced H_2O_2 production requires the activation of phosphatidylinositol 3-kinase. J Biol Chem 2000; 275: 10527–31.

65 Li Q, Spencer NY, Oakley FD, Buettner GR, Engelhardt JF. Endosomal Nox2 facilitates redox-dependent induction of NF-κB by TNF-α. Antioxid Redox Signal 2009; 11: 1249–63.

66 Li JM, Fan LM, George VT, Brooks G. Nox2 regulates endothelial cell cycle arrest and apoptosis via p21cip1 and p53. Free Radic Biol Med 2007; 43: 976–86.

67 Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci U S A 1991; 88: 10540–3.

68 Jolitha AB, Subramanyam MVV, Asha Devi S. Modification by vitamin E and exercise of oxidative stress in regions of aging rat brain: studies on superoxide dismutase isoenzymes and protein oxidation status. Exp Gerontol 2006; 41: 753–63.

69 Perluigi M, Di Domenico F, Giorgi A, Schininà ME, Coccia R, Cini C, Bellia F, Cambria MT, Cornelius C, Butterfield DA, Calabrese V. Redox proteomics in aging rat brain: involvement of mitochondrial reduced glutathione status and mitochondrial protein oxidation in the aging process. J Neurosci Res 2010; 88: 3498–507.

70 Dei R, Takeda A, Niwa H, Li M, Nakagomi Y, Watanabe M, Inagaki T, Washimi Y, Yasuda Y, Horie K, Miyata T, Sobue G. Lipid peroxidation and advanced glycation end products in the brain in normal aging and in Alzheimer's disease. Acta Neuropathol 2002; 104: 113–22.

71 Leutner S, Eckert A, Müller WE. ROS generation, lipid peroxidation and antioxidant enzyme activities in the aging brain. J Neural Transm 2001; 108: 955–67.

72 Clausen A, Doctrow S, Baudry M. Prevention of cognitive deficits and brain oxidative stress with superoxide dismutase/catalase mimetics in aged mice. Neurobiol Aging 2010; 31: 425–33.

73 Nicolle MM, Gonzalez J, Sugaya K, Baskerville KA, Bryan D, Lund K, Gallagher M, McKinney M. Signatures of hippocampal oxidative stress in aged spatial learning-impaired rodents. Neuroscience 2001; 107: 415–31.

74 Quick KL, Ali SS, Arch R, Xiong C, Wozniak D, Dugan LL. A carboxyfullerene SOD mimetic improves cognition and extends the lifespan of mice. Neurobiol Aging 2008; 29: 117–28.

75 Park L, Anrather J, Girouard H, Zhou P, Iadecola C. Nox2-derived reactive oxygen species mediate neurovascular dysregulation in the aging mouse brain. J Cereb Blood Flow Metab 2007; 27: 1908–18.

76 Venkateshappa C, Harish G, Mythri RB, Mahadevan A, Bharath MMS, Shankar SK. Increased oxidative damage and decreased antioxidant function in aging human substantia nigra compared to striatum: implications for Parkinson's disease. Neurochem Res 2012; 37: 358–69.

77 Van der Loo B, Bachschmid M, Spitzer V, Brey L, Ullrich V, Lüscher TF. Decreased plasma and tissue levels of vitamin C in a rat model of aging: implications for antioxidative defense. Biochem Biophys Res Commun 2003; 303: 483–7.

78 Van der Loo B, Labugger R, Aebischer CP, Bachschmid M, Spitzer V, Kilo J, Altwegg L, Ullrich V, Lüscher TF. Age-related changes of vitamin A status. J Cardiovasc Pharmacol 2004; 43: 26–30.

79 Morrison CD, Pistell PJ, Ingram DK, Johnson WD, Liu Y, Fernandez-Kim SO, White CL, Purpera MN, Uranga RM, Bruce-Keller AJ, Keller JN. High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling. J Neurochem 2010; 114: 1581–9.

80 Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2001; 56: 1133–42.

81 Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999; 56: 303–8.

82 Farias ST, Mungas D, Reed BR, Harvey D, DeCarli C. Progression of mild cognitive impairment to dementia in clinic- *vs.* community-based cohorts. Arch Neurol 2009; 66: 1151–7.

83 Yesavage JA, O'Hara R, Kraemer H, Noda A, Taylor JL, Ferris S, Gély-Nargeot M-C, Rosen A, Friedman L, Sheikh J, Derouesné C. Modeling the prevalence and incidence of Alzheimer's disease and mild cognitive impairment. J Psychiatr Res 2002; 36: 281–6.

84 Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 2005; 64: 1152–6.

85 Torres LL, Quaglio NB, de Souza GT, Garcia RT, Dati LMM, Moreira WL, Loureiro AP, de Souza-Talarico JN, Smid J, Porto CS, Bottino CM, Nitrini R, Barros SB, Camarini R, Marcourakis T. Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 2011; 26: 59–68.

86 Baldeiras I, Santana I, Proença MT, Garrucho MH, Pascoal R, Rodrigues A, Duro D, Oliveira CR. Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease. J Alzheimers Dis 2008; 15: 117–28.

87 López N, Tormo C, De Blas I, Llinares I, Alom J. Oxidative stress in Alzheimer's disease and mild cognitive impairment with high sensitivity and specificity. J Alzheimers Dis 2013; 33: 823–9.

88 Praticò D, Clark CM, Liun F, Rokach J, Lee VY-M, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol 2002; 59: 972–6.

89 Lee LK, Shahar S, Rajab N, Yusoff NAM, Jamal RA, Then SM. The role of long chain omega-3 polyunsaturated fatty acids in reducing lipid peroxidation among elderly patients with mild cognitive impairment: a case-control study. J Nutr Biochem 2013; 24: 803–8.

90 Butterfield DA, Reed T, Perluigi M, De Marco C, Coccia R, Cini C, Sultana R. Elevated protein-bound levels of the lipid peroxidation product, 4-hydroxy-2-nonenal, in brain from persons with mild cognitive impairment. Neurosci Lett 2006; 397: 170–3.

91 Butterfield DA, Reed TT, Perluigi M, De Marco C, Coccia R, Keller JN, Markesbery WR, Sultana R. Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. Brain Res 2007; 1148: 243–8.

92 Cha H-N, Kim Y-W, Kim J-Y, Kim Y-D, Song IH, Min K-N, Park S-Y. Lack of inducible nitric oxide synthase does not prevent aging-associated insulin resistance. Exp Gerontol 2010; 45: 711–8.

93 Guidi I, Galimberti D, Lonati S, Novembrino C, Bamonti F, Tiriticco M, Fenoglio C, Venturelli E, Baron P, Bresolin N, Scarpini E. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 2006; 27: 262–9.

94 Rinaldi P, Polidori MC, Metastasio A, Mariani E, Mattioli P, Cherubini A, Catani M, Cecchetti R, Senin U, Mecocci P. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003; 24: 915–9.

95 Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. Neurosci Lett 2010; 469: 6–10.

96 Dugan LL, Ali SS, Shekhtman G, Roberts AJ, Lucero J, Quick KL, Behrens MM. IL-6 mediated degeneration of forebrain GABAergic interneurons and cognitive impairment in aged mice through activation of neuronal NADPH oxidase. PLoS ONE 2009; 4: e5518.

97 Bruce-Keller AJ, Gupta S, Parrino TE, Knight AG, Ebenezer PJ, Weidner AM, LeVine H, Keller JN, Markesbery WR. NOX activity is increased in mild cognitive impairment. Antioxid Redox Signal 2010; 12: 1371–82.

98 Park L, Zhou P, Pitstick R, Capone C, Anrather J, Norris EH, Younkin L, Younkin S, Carlson G, McEwen BS, Iadecola C. Nox2-derived radicals contribute to neurovascular and behavioral dysfunction in mice overexpressing the amyloid precursor protein. Proc Natl Acad Sci U S A 2008; 105: 1347–52.

99 Ali SS, Young JW, Wallace CK, Gresack J, Jeste DV, Geyer MA, Dugan LL, Risbrough VB. Initial evidence linking synaptic superoxide production with poor short-term memory in aged mice. Brain Res 2011; 1368: 65–70.

100 Bruce-Keller AJ, Gupta S, Knight AG, Beckett TL, McMullen JM, Davis PR, Murphy MP, Van Eldik LJ, St Clair D, Keller JN. Cognitive impairment in humanized APP × PS1 mice is linked to $A\beta_{1-42}$ and NOX activation. Neurobiol Dis 2011; 44: 317–26.

101 Ansari MA, Scheff SW. NADPH-oxidase activation and cognition in Alzheimer disease progression. Free Radic Biol Med 2011; 51: 171–8.

102 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition: DSM-IV-TR®. Washington DC: American Psychiatric Association, 2000.

103 McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7: 263–9.

104 Ittner LM, Götz J. Amyloid-β and tau – a toxic *pas de deux* in Alzheimer's disease. Nat Rev Neurosci 2011; 12: 65–72.

105 Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T, Hayakawa T, Nunomura A, Chiba S, Perry G, Smith MA, Fujimoto S. Activation of NADPH oxidase in Alzheimer's disease brains. Biochem Biophys Res Commun 2000; 273: 5–9.

106 Zekry D, Epperson TK, Krause K-H. A role for NOX NADPH oxidases in Alzheimer's disease and other types of dementia? IUBMB Life 2003; 55: 307–13.

107 Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD. Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. JAMA 2000; 283: 1571–7.

108 Carrano A, Hoozemans JJM, van der Vies SM, Rozemuller AJM, van Horssen J, de Vries HE. Amyloid Beta induces oxidative stress-mediated blood-brain barrier changes in capillary amyloid angiopathy. Antioxid Redox Signal 2011; 15: 1167–78.

109 Carrano A, Hoozemans JJM, van der Vies SM, van Horssen J, de Vries HE, Rozemuller AJM. Neuroinflammation and blood-brain barrier changes in capillary amyloid angiopathy. Neurodegener Dis 2012; 10: 329–31.

110 Heumüller S, Wind S, Barbosa-Sicard E, Schmidt HHHW, Busse R, Schröder K, Brandes RP. Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. Hypertension 2008; 51: 211–7.

111 Stolk J, Hiltermann TJ, Dijkman JH, Verhoeven AJ. Characteristics of the inhibition of NADPH oxidase activation in neutrophils by apocynin, a methoxy-substituted catechol. Am J Respir Cell Mol Biol 1994; 11: 95–102.

112 Ximenes VF, Kanegae MPP, Rissato SR, Galhiane MS. The oxidation of apocynin catalyzed by myeloperoxidase: proposal for NADPH oxidase inhibition. Arch Biochem Biophys 2007; 457: 134–41.

113 Touyz RM. Apocynin, NADPH oxidase, and vascular cells: a complex matter. Hypertension 2008; 51: 172–4.

114 Johnson DK, Schillinger KJ, Kwait DM, Hughes CV, McNamara EJ, Ishmael F, O'Donnell RW, Chang M-M, Hogg MG, Dordick JS, Santhanam L, Ziegler LM, Holland JA. Inhibition of NADPH oxidase activation in endothelial cells by ortho-methoxy-substituted catechols. Endothelium 2002; 9: 191–203.

115 Dumont M, Stack C, Elipenhali C, Calingasan NY, Wille E, Beal MF. Apocynin administration does not improve behavioral and neuropathological deficits in a transgenic mouse model of Alzheimer's disease. Neurosci Lett 2011; 492: 150–4.

116 Wang Q, Tompkins KD, Simonyi A, Korthuis RJ, Sun AY, Sun GY. Apocynin protects against global cerebral ischemia-reperfusion-induced oxidative stress and injury in the gerbil hippocampus. Brain Res 2006; 1090: 182–9.

117 Harraz MM, Marden JJ, Zhou W, Zhang Y, Williams A, Sharov VS, Nelson K, Luo M, Paulson H, Schöneich C, Engelhardt JF. SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. J Clin Invest 2008; 118: 659–70.

118 Qin B, Cartier L, Dubois-Dauphin M, Li B, Serrander L, Krause K-H. A key role for the microglial NADPH oxidase in APP-dependent killing of neurons. Neurobiol Aging 2006; 27: 1577–87.

119 Wilkinson B, Koenigsknecht-Talboo J, Grommes C, Lee CYD, Landreth G. Fibrillar β-amyloid-stimulated intracellular signaling cascades require Vav for induction of respiratory burst and phagocytosis in monocytes and microglia. J Biol Chem 2006; 281: 20842–50.

120 Surace MJ, Block ML. Targeting microglia-mediated neurotoxicity: the potential of NOX2 inhibitors. Cell Mol Life Sci 2012; 69: 2409–27.

121 Shelat PB, Chalimoniuk M, Wang J-H, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY. Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. J Neurochem 2008; 106: 45–55.

122 Sun GY, Horrocks LA, Farooqui AA. The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory responses in neurodegenerative diseases. J Neurochem 2007; 103: 1–16.

123 Hensley K, Floyd RA, Zheng NY, Nael R, Robinson KA, Nguyen X, Pye QN, Stewart CA, Geddes J, Markesbery WR, Patel E, Johnson GV, Bing G. p38 kinase is activated in the Alzheimer's disease brain. J Neurochem 1999; 72: 2053–8.

124 De la Monte SM, Wands JR. Molecular indices of oxidative stress and mitochondrial dysfunction occur early and often progress with severity of Alzheimer's disease. J Alzheimers Dis 2006; 9: 167–81.

125 Bodles AM, Barger SW. Secreted β-amyloid precursor protein activates microglia via JNK and p38-MAPK. Neurobiol Aging 2005; 26: 9–16.

126 Lee SY, Lee JW, Lee H, Yoo HS, Yun YP, Oh KW, Ha TY, Hong JT. Inhibitory effect of green tea extract on

β-amyloid-induced PC12 cell death by inhibition of the activation of NF-κB and ERK/p38 MAP kinase pathway through antioxidant mechanisms. Brain Res Mol Brain Res 2005; 140: 45–54.

127 Valerio A, Boroni F, Benarese M, Sarnico I, Ghisi V, Bresciani LG, Ferrario M, Borsani G, Spano P, Pizzi M. NF-κB pathway: a target for preventing β-amyloid (Aβ)-induced neuronal damage and Aβ42 production. Eur J Neurosci 2006; 23: 1711–20.

128 Calabresi P, Castrioto A, Di Filippo M, Picconi B. New experimental and clinical links between the hippocampus and the dopaminergic system in Parkinson's disease. Lancet Neurol 2013; 12: 811–21.

129 Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 2008; 79: 368–76.

130 Harrigan TJ, Abdullaev IF, Jourd'heuil D, Mongin AA. Activation of microglia with zymosan promotes excitatory amino acid release via volume-regulated anion channels: the role of NADPH oxidases. J Neurochem 2008; 106: 2449–62.

131 Zhang W, Wang T, Qin L, Gao H-M, Wilson B, Ali SF, Zhang W, Hong J-S, Liu B. Neuroprotective effect of dextromethorphan in the MPTP Parkinson's disease model: role of NADPH oxidase. FASEB J 2004; 18: 589–91.

132 Qin L, Liu Y, Hong J-S, Crews FT. NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic neurodegeneration following systemic LPS administration. Glia 2013; 61: 855–68.

133 Wu D-C, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, Przedborski S. NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. Proc Natl Acad Sci U S A 2003; 100: 6145–50.

134 Huh SH, Chung YC, Piao Y, Jin MY, Son HJ, Yoon NS, Hong JY, Pak YK, Kim YS, Hong JK, Hwang O, Jin BK. Ethyl pyruvate rescues nigrostriatal dopaminergic neurons by regulating glial activation in a mouse model of Parkinson's disease. J Immunol 2011; 187: 960–9.

135 Liang L-P, Huang J, Fulton R, Day BJ, Patel M. An orally active catalytic metalloporphyrin protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in vivo. J Neurosci 2007; 27: 4326–33.

136 Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, Zhou C, Geghman K, Bogdanov M, Przedborski S, Beal MF, Burke RE, Li C. Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat Neurosci 2009; 12: 826–8.

137 Dranka BP, Gifford A, Ghosh A, Zielonka J, Joseph J, Kanthasamy AG, Kalyanaraman B. Diapocynin prevents early Parkinson's disease symptoms in the leucine-rich repeat kinase 2 (LRRK2(R1441G)) transgenic mouse. Neurosci Lett 2013; 549: 57–62.

138 Chongthammakun V, Sanvarinda Y, Chongthammakun S. Reactive oxygen species production and MAPK activation are implicated in tetrahydrobiopterin-induced SH-SY5Y cell death. Neurosci Lett 2009; 449: 178–82.

139 Newhouse K, Hsuan S-L, Chang SH, Cai B, Wang Y, Xia Z. Rotenone-induced apoptosis is mediated by p38 and JNK MAP kinases in human dopaminergic SH-SY5Y cells. Toxicol Sci 2004; 79: 137–46.

140 Ferrer I, Blanco R, Carmona M, Puig B, Barrachina M, Gómez C, Ambrosio S. Active, phosphorylation-dependent mitogen-activated protein kinase (MAPK/ERK), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and p38 kinase expression in Parkinson's disease and Dementia with Lewy bodies. J Neural Transm 2001; 108: 1383–96.

141 Saporito MS, Thomas BA, Scott RW. MPTP activates c-Jun NH_2-terminal kinase (JNK) and its upstream regulatory kinase MKK4 in nigrostriatal neurons in vivo. J Neurochem 2000; 75: 1200–8.

142 Karunakaran S, Diwakar L, Saeed U, Agarwal V, Ramakrishnan S, Iyengar S, Ravindranath V. Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by α-lipoic acid. FASEB J 2007; 21: 2226–36.

143 Thomas T, Timmer M, Cesnulevicius K, Hitti E, Kotlyarov A, Gaestel M. MAPKAP kinase 2-deficiency prevents neurons from cell death by reducing neuroinflammation – relevance in a mouse model of Parkinson's disease. J Neurochem 2008; 105: 2039–52.

144 Karunakaran S, Saeed U, Mishra M, Valli RK, Joshi SD, Meka DP, Seth P, Ravindranath V. Selective activation of p38 mitogen-activated protein kinase in dopaminergic neurons of substantia nigra leads to nuclear translocation of p53 in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice. J Neurosci 2008; 28: 12500–9.

145 Duan W, Zhu X, Ladenheim B, Yu Q, Guo Z, Oyler J, Cutler RG, Cadet JL, Greig NH, Mattson MP. p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism. Ann Neurol 2002; 52: 597–606.

146 Wang H-M, Wang L-W, Liu X-M, Li C-L, Xu S-P, Farooq A-D. Neuroprotective effects of forsythiaside on learning and memory deficits in senescence-accelerated mouse prone (SAMP8) mice. Pharmacol Biochem Behav 2013; 105: 134–41.

147 Farr SA, Poon HF, Dogrukol-Ak D, Drake J, Banks WA, Eyerman E, Butterfield DA, Morley JE. The antioxidants α-lipoic acid and *N*-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. J Neurochem 2003; 84: 1173–83.

148 Liu R, Liu IY, Bi X, Thompson RF, Doctrow SR, Malfroy B, Baudry M. Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. Proc Natl Acad Sci U S A 2003; 100: 8526–31.

149 Raghavendra V, Kulkarni SK. Possible antioxidant mechanism in melatonin reversal of aging and chronic

ethanol-induced amnesia in plus-maze and passive avoidance memory tasks. Free Radic Biol Med 2001; 30: 595–602.

150 Praticò D, Uryu K, Leight S, Trojanoswki JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J Neurosci 2001; 21: 4183–7.

151 Ghosh A, Kanthasamy A, Joseph J, Anantharam V, Srivastava P, Dranka BP, Kalyanaraman B, Kanthasamy AG. Anti-inflammatory and neuroprotective effects of an orally active apocynin derivative in pre-clinical models of Parkinson's disease. J Neuroinflammation 2012; 9: 241.

152 Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, Hartley DM, Ghosh S, Mosley RL, Gendelman HE, Pahan K. Selective inhibition of NF-κB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proc Natl Acad Sci U S A 2007; 104: 18754–9.

153 Butterfield DA, Poon HF, St Clair D, Keller JN, Pierce WM, Klein JB, Markesbery WR. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. Neurobiol Dis 2006; 22: 223–32.

154 Sultana R, Poon HF, Cai J, Pierce WM, Merchant M, Klein JB, Markesbery WR, Butterfield DA. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. Neurobiol Dis 2006; 22: 76–87.

155 Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. J Neurochem 2005; 93: 953–62.

156 Castegna A, Thongboonkerd V, Klein JB, Lynn B, Markesbery WR, Butterfield DA. Proteomic identification of nitrated proteins in Alzheimer's disease brain. J Neurochem 2003; 85: 1394–401.

157 Isobe C, Abe T, Terayama Y. Levels of reduced and oxidized coenzyme Q-10 and 8-hydroxy-2′-deoxyguanosine in the cerebrospinal fluid of patients with living Parkinson's disease demonstrate that mitochondrial oxidative damage and/or oxidative DNA damage contributes to the neurodegener. Neurosci Lett 2010; 469: 159–63.

158 White LR, Toft M, Kvam SN, Farrer MJ, Aasly JO. MAPK-pathway activity, Lrrk2 G2019S, and Parkinson's disease. J Neurosci Res 2007; 85: 1288–94.

159 Prigione A, Begni B, Galbussera A, Beretta S, Brighina L, Garofalo R, Andreoni S, Piolti R, Ferrarese C. Oxidative stress in peripheral blood mononuclear cells from patients with Parkinson's disease: negative correlation with levodopa dosage. Neurobiol Dis 2006; 23: 36–43.

160 Serra JA, Domínguez RO, de Lustig ES, Guareschi EM, Famulari AL, Bartolomé EL, Marschoff ER. Parkinson's disease is associated with oxidative stress: comparison of peripheral antioxidant profiles in living Parkinson's, Alzheimer's and vascular dementia patients. J Neural Transm 2001; 108: 1135–48.